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Involvement of Voltage-Gated Sodium Channels in Breast Cancer Cells Invasiveness and Regulation by Docosahexaenoic Acid
Ludovic Gillet, Sébastien Roger, Sarah Calaghan, Philippe Bougnoux, Pierre Besson, Jean-Yves Le Guennec.
Voltage-gated sodium channels (Na_V) are expressed in highly invasive metastatic cancer cells derived from epithelial tissues. In the highly invasive breast cancer cell line MDA-MB-231, we showed that the abnormal expression of $Na_V1.5$ was responsible for a sustained inward sodium current at the membrane potential, which promotes the invasion of the extracellular matrix. We found that $Na_V1.5$ activity leads to a perimembrane acidification, favourable for the pH-dependent proteolytic activity of cysteine cathepsins which play a predominant role in cell invasiveness. Moreover, Na_V and the sodium-proton exchanger NHE1 (a pH regulator) seem to be colocalized in specialized lipid rafts domains, caveolae, where cathepsins-B are known to be secreted.
Since docosahexaenoic acid (DHA, 22:6 n-3) has been reported to reduce the aggressiveness of some breast cancers, and to be a modulator of ion channels, we investigated its role on Na_V activity and cell invasiveness. Supplementation of MDA-MB-231 cells culture medium with 1 μM DHA for five days affected neither cell migration nor proliferation but significantly reduced cell invasiveness, while oleic acid (OA, 18:1 n-9), used as a control, had no effect. Moreover, the combination of DHA with tetrodotoxin (specific Na_V blocker) demonstrated no further effect on invasion, suggesting a possible regulation of Na_V activity by DHA. Indeed, Na_V currents were reduced with DHA but not with OA supplementation. These fatty acids were inefficient to reduce sodium currents when acutely applied, suggesting a middle- or long-term regulation of Na_V channels. However, no effect on Na_V mRNA or protein expression and no change in Na_V raft localization was observed.
Thus, DHA effects could occur through a modification in cholesterol/phospholipids composition of the plasma membrane and/or a modification in factors regulating Na_V channels activity and/or addressing to the plasma membrane.

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Endplate Na^+ Channels Loss Impairs Neuromuscular Transmission in Myasthenia Gravis
Robert Louis Ruff.
Myasthenia gravis (MG) is caused by acetylcholine receptor (AChR) antibodies inducing complement-medicated destruction of endplate membrane. Endplate Na^+ channels enhance the safety factor for neuromuscular transmission by reducing the threshold depolarization needed to trigger an action potential (E_{AP}). I previously demonstrated that MG antibodies do not directly alter the function of Na^+ channels and that Na+ channels are lost by complement-mediated loss of endplate membrane. I examined the impact of postsynaptic voltage-gated Na^+ channel loss on the SF. Comparing intercostal nerve-muscle preparations from controls and MG patients with moderate (M) or severe (S) MG, I found that endplate AChR loss decreased the endplate potential (EPP) from 40.2 ±1.3 to 30.1±1.3 mV (M) and 23.5 ±1.7 (S) ($p < 0.001$ for both). Endplate Na+ channel loss increased E_{AP} from −71.9 ±2.2 to −66.1 ±2.7 (M; $p < 0.01$) and 62.3 ±2.7 mV (S; $p < 0.001$). In contrast, on extrajunctional membrane (> 0.1 cm from the endplate), E_{AP} was the same for MG fibers and controls. The SF for neuromuscular transmission was reduced from 2.98 to 1.58 (M) and 1.09 (S). If the EPP was the same as control, the SF for MG fibers would have been 2.12 (M) or 1.86 (S). If E_{AP} for MG fibers were the same as control fibers, the SF for MG fibers would have been 2.23 (M) or 1.74(S). In moderate MG, reduced EPP accounted for 47% of the SF reduction and increased E_{AP} 53% of the SF reduction. In severe MG, reduced EPP accounted for 59% of the SF reduction and increased E_{AP} 40% of the SF reduction. Conclusion: loss of endplate Na^+ channels in MG contributes to the reduction in SF to a similar extent as does the loss of endplate AChRs.

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Molecular Characterisation of the Voltage-Gated Sodium Channel from Aphids
Joanna S. Amey, Mark J. Burton, Andrias O. O'Reilly, Martin S. Williamson, Ian R. Mellor, B.A. Wallace, Linda M. Field, T.G.E. Davies.
We describe the gene structure and primary sequence of the voltage-gated sodium channel (VGSC) of *Acyrthosiphon pisum* (Harris) and the characterisation of the corresponding VGSC from the closely-related aphid *Myzuspersicae* (Sulzer), an economically important crop pest. The *A. pisum* sodium channel sequence spans more than 46 Kb on a single scaffold of the recently-released second assembly of the pea aphid genome. In-silico analysis of the VGSC suggests that channel domains I and II are encoded separately from domains III and IV on two different genes. Northern and Southern blots of *A. pisum* genomic material are currently being employed to confirm our findings.
The aphid genes show a high degree of sequence homology to VGSCs from other species, although closer analysis of the pore region reveals an unusual selectivity filter within the P-loops of the aphid channels consisting of DENS (domains I-IV) rather than the standard sodium selective DEKA filter. Site-directed mutagenesis of the *Drosophila melanogaster* 'para' VGSC was undertaken to recreate part or all of the aphid selectivity filter and subsequent expression of these modified channels in *Xenopus* oocytes yielded voltage-dependent currents with unusual properties. Homology modelling and ligand docking studies suggested that the aphid VGSCs may be insensitive to TTX. We have confirmed this prediction experimentally using both bioassay studies and voltage-clamp recordings of oocytes expressing 'para' channels modified within the TTX binding site. This is the first report of a VGSC encoded by two separate genes and containing an alternative selectivity filter.

Voltage-Gated K Channels - Gating II

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FRET-Based Assessments of Interactions Between KCNQ1 and HERG Potassium Channel α-Subunits
Louise E. Organ-Darling, Amanda N. Vernon, Gong-Xin Liu, Gideon Koren.
Voltage-gated potassium channels play a critical role in repolarizing cardiomyocytes, and abnormal repolarization leads to prolonged action potential durations (APDs), which manifest clinically as Long-QT (LQT) syndrome and can result in malignant arrhythmias and sudden cardiac death. LQT1 is due to mutations in KCNQ1 (KvlQT1) α-subunits which underlie the cardiac I_{Ks} current while LQT2 results from reductions in I_{Kr} which is carried by KCNH2 (HERG) channels. I_{Kr} and I_{Ks} appear interconnected in normal hearts and function together to maintain appropriate APDs. However, electrophysiological studiesin LQT1 and LQT2 transgenic rabbits as well as in stable cell lines demonstrated downregulation of the reciprocal repolarizing currents. Cells overexpressing pore mutants of KCNQ1, and therefore lacking I_{Ks}, show decreased I_{Kr} current while cells overexpressing HERG pore mutants, having reduced I_{Kr}, show downregulated I_{Ks}. Direct interactions between the C-termini of KCNQ1 and HERG have been shown by surface plasmon resonance assays (Ren et al., 2010, PMID: 20833965). To further examine the interaction between KCNQ1 and HERG we have developed and characterized a library of fluorescent protein fusion constructs to utilize in quantitative microscopy experiments. We hypothesize that functionally relevant, dynamic interactions between KCNQ1 and HERG occur within the plasma membrane. We will test this hypothesis with several FRET-based assays aimed at demonstrating measureable interactions between KCNQ1 and HERG and establishing a foundation to study the dynamics of such interactions.

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Mechanistic Insight into hERG Channel Deactivation Gating from the Solution Structure of the eag Domain
Frederick Muskett, **Steven Thomson**, Samrat Thouta, Alexander Bowen, Phillip Stansfeld, John Mitcheson.
hERG (Kv11.1) potassium channels deactivate slowly and this is critical for regulating the time course and amplitude of repolarising current during the cardiac action potential. Inherited mutations that alter deactivation and reduce hERG current are linked to long QT syndrome, a potentially lethal type of cardiac arrhythmia. The N-terminus is known to have a key role in regulating the slow deactivation and its removal dramatically accelerates deactivation. However, the precise molecular mechanism remains poorly understood. Deletion of residues 2 to 26 accelerates deactivation to a similar extent as removing the whole of the N-terminus. Slow deactivation can be restored to the N-truncated channel by the addition of a soluble peptide corresponding to residues 2-16. Residues 1 - 135 form the 'eag domain' which is conserved in the ether-a-go-go family of potassium channels. A crystal structure for part of the eag domain (Arg27 - Lys135) of hERG has been solved and shown to contain a *PAS* domain, however the functionally critical first 26 residues (NT1-26) were not resolved. Here we present an NMR solution structure of the whole of the eag domain. This new structure reveals a previously undiscovered amphipathic α-helix from residues Gln11 to Gly24, while the first 10 residues are extended and highly dynamic. Electrophysiological and mutagenesis data show that the helix is functionally important. Breaking it by Pro substitution accelerated deactivation to rates similar to the N-truncated channel. One face of NT1-26 is positively charged. Alanine substitution of basic residues (R4A:R5A and R20A:K21A) also profoundly accelerates hERG current deactivation. Our results indicate that slow deactivation of hERG involves NT1-26 binding to the channel via electrostatic interactions that stabilise the open conformation of the pore.